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On-line monitoring of water quality with a floating microbial fuel cell biosensor: field test results

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Abstract

Real-time biomonitoring using microbial fuel cell (MFC) based biosensors have been demonstrated in several laboratory studies, but field validation is lacking. This study describes the long-term performance of an MFC based biosensor developed for real-time monitoring of changes in the water quality of a metal-contaminated stream. After a startup in the laboratory, biosensors were deployed in a stream close to an active mining complex in Sudbury, ON, Canada. Three sites within the stream were selected for biosensors installation based on their positions relative to the mining complex discharge points - upstream (lowest heavy metals concentration), midpoint and downstream. The biosensors installed at these sites were able to detect, in real-time, temporal changes in the water quality over a 2-month period. The biosensor response was confirmed by the results of a conventional toxicity assay (48-h acute *Daphnia magna*) as well as analytical measurements of heavy metals concentration in the stream. We conclude that the biosensor could detect changes in the overall water quality of the stream despite the uncontrolled situations typical for field operations as compared to laboratory conditions. To further explain the results observed during the field test, the rapid Microtox bioassay and *D. magna* assay were used to investigate the possible contributions of the two dominant mining metals (Nickel and Copper) to water toxicity in the test area.

Keywords Biomonitoring · Biosensor · Toxicity · On-line · Microbial fuel cell · Aquatic toxicity

Introduction

One of the most persistent and complex environmental issues stems from the contamination from active and closed mining sites. Heavy metal ions from the mining process can accumulate in aquatic ecosystems and communities (Herlory et al. 2013), worsening water quality and leading to mortality in several organisms. The toxicity effect can be perpetuated as heavy metals also accumulate in sediments, leading to a surge-release during the dry/wet or winter/spring interface (Besley and Chessman 2008; Nriagu et al. 1998). In particular, the monitoring of toxicity of trace metals such as Cu, Ni, Pb, etc (usually present at less than 0.01% dry weight in living organisms) is important because they are commercially mined. Unfortunately, traditional monitoring methods, e.g. *Daphnia magna* lethality tests, Microtox, microalgae, and fish assays, are laboratory based. Results obtained under controlled laboratory conditions may not translate to field situations. Several factors such as temperature, physiological acclimation, water chemistry and interactions between various dissolved metals must be taken into consideration in reviewing analytical results (Brezonik et al. 1991; Erickson et al. 1996; Hyne et al. 2005). Traditional monitoring methods are also typically conducted using a single grab sample that does not reflect temporal variability in water quality. Other challenges of these methods include high costs, long testing time, ethical considerations and logistic problems relating to sample collection and transportation to the laboratory.

Biosensors based on bioelectrochemical systems are being developed as an alternative fast and inexpensive water quality monitoring method. In brief, bioelectrochemical systems employ electroactive microorganisms for the oxidation of organic materials at the anode and a reduction

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reaction at the cathode (Debabov 2008). Therefore, interference in the redox reaction e.g. by heavy metal ions, are reflected in the measured voltage or current of the system. Notably, microbial fuel cells (MFCs) are the most developed bioelectrochemical systems for biosensing. The mechanism of heavy metals interference and subsequent toxicity detection in MFC biosensors is detailed in several laboratory studies (Adekunle et al. 2019; Kim et al. 2010; Stein et al. 2011; Sun et al. 2015; Yu et al. 2017; Zhou et al. 2017; Zhu and Logan 2014). These biosensors are inexpensive, and easy to maintain (Adekunle et al. 2020; Jiang et al. 2018; Jiang et al. 2015; Yi et al. 2019; Yu et al. 2017). Yet, successful field applications, which solve the most challenging problems associated with current traditional monitoring methods, site specificity and sampling data frequency, are lacking. To date, only one study (Adekunle et al. 2020), has attempted real-time water quality monitoring in the field using MFC-based biosensors.

This work evaluates the field performance of an MFC-based biosensor, capable of detecting toxicity related to heavy metals, for real-time water quality monitoring. A potential application area identified for the biosensor was mining watershed, where water quality is closely related to heavy metals mined from ore deposits. Therefore, the field component of this work was carried out in the Junction Creek, Sudbury, ON, Canada watershed where Cu and Ni are being mined commercially. Firstly, to achieve long-term field operation, the biosensor was modified by introducing a slow-release carbon source, which improved organic matter availability and reduced exposure of the anodic biofilm to dissolved oxygen. Thereafter, the biosensor's long-term performance is evaluated in three different locations within the Junction Creek watershed for up to 8 weeks. Each of the locations had historically distinct heavy metals concentration profiles. *Daphnia magna* toxicity assays are simultaneously carried out to provide a basis for comparison with the biosensor performance. Furthermore, to validate biosensor results, Microtox bioassays are carried out to investigate the possible contributions of the two dominant mining metals (Nickel and Copper) to the observed water quality differences.

Materials and methods

Analytical methods and stock solutions

During the startup and maintenance, the biosensors were operated on moderately hard water (MHW) composed of (in mg L^{-1}): CaSO4 (60), MgSO4 (122.86), NaHCO3 (96), and KCl (4). When needed for the Microtox assay and *D. magna* assay, a stock solution containing 80 mg L^{-1} of Ni and 8 mg L^{-1} of Cu prepared in MHW was diluted with more MHW.

The concentrations (dissolved and total) of heavy metals at the biosensor deployment sites were determined by inductive coupled plasma mass spectrophotometry (ICP-MS), as described elsewhere (Beauchemin 2010). Total metals concentrations were measured in 15 mL acidified (with 1% HNO_3) water samples, while 15 mL filtered (<0.45 µm) water samples were used for determination of dissolved metals concentration.

Biosensors design and operation

The rectangular-shaped floating biosensors used in this study were printed on a 3D printer from polylactide (PLA) according to the design detailed in Adekunle et al. (2020). Slits at the bottom of the biosensor frame allowed for water movement through the biosensor. The anode in the floating biosensor was 10 mm thick and made of two layers of 10 × 5 cm carbon felt (SGL Group, Charlotte, NC, USA). 5 mL of birch sawdust (1–3 mm in length) was added below the carbon felt to provide carbon supply and to reduce exposure of the microbial biofilm to dissolved oxygen. The air cathode (Electric Fuel Ltd, Bet Shemesh, Israel) was 10 × 5 cm. Flotation of the biosensor was maintained using a hollow plastic ring filled with Styrofoam and the biosensor was anchored in place at least one meter away from the shore at each deployment site. Figure 1A shows the 3D representation of the floating MFC-based biosensor and the floatation mechanism.

During field tests, biosensor voltage and water temperature values were acquired every 15 min. The biosensor voltage is measured under steady state conditions when an external resistor (R_{ext}) is either connected or disconnected, as described in previous studies (Adekunle et al. 2019; Adekunle et al. 2020). As shown in these studies, changes in the water quality are reflected in the V_{max} (open-circuit voltage of the biosensor), and V_{min} (voltage during biosensor connection to the R_{ext}) values of the biosensor. In general, these biosensor outputs (V_{max} and V_{min}) decrease with increasing toxicity and vice versa. In this study, the V_{min} output of the biosensor was the output critically analyzed as it has been shown to be more responsive to toxicity changes than V_{max} (Adekunle et al. 2019).

A 5 kOhm R_{ext} was used in this study and the R_{ext} connection/disconnection was achieved using G6S-2F relays (Omron LLS, IL, USA). The control algorithm for the biosensor outputs measurement was implemented in Python (Python https://www.python.org/) on a Raspberry Pi-3 Model B single-board computer. LabJack U3-LV (LabJack Corp., Lakewood, CO, USA) was used for data

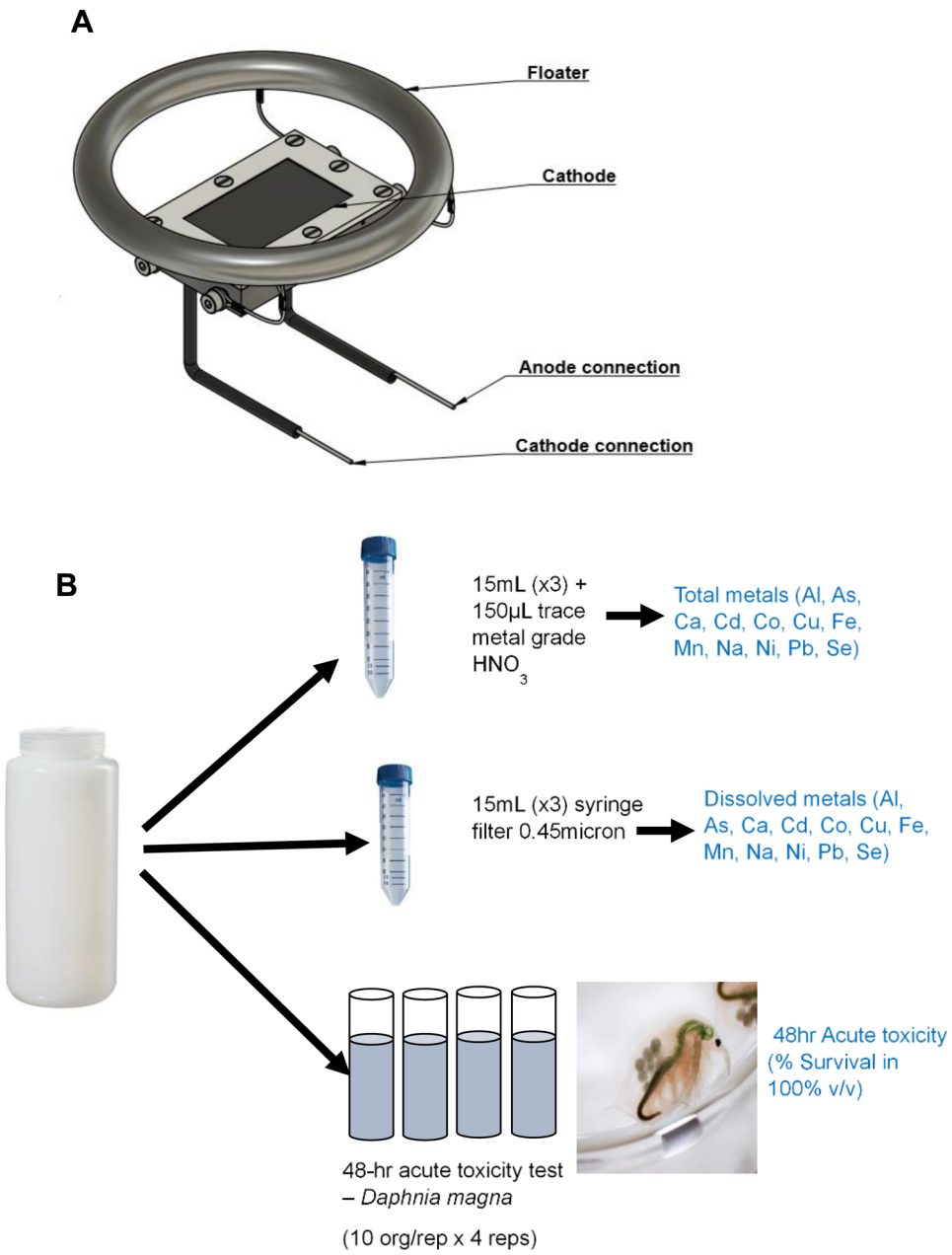

Fig. 1 Water quality monitoring experimental setups. **A** 3D representation of the floating biosensor and (**B**), sample collection for analyses and Daphnia assay

acquisition. Labjack devices were connected to the Pi-3's with a USB cable for data exchange and power. According to the control algorithm, the Python program periodically requests voltage readings from the LabJack channels connected to the MFC and to a temperature sensor. The MFC voltage is used for calculating the V_{max} and V_{min} as detailed in our previous study (Adekunle et al. 2019). For the field test, the Pi-3 computers and the data acquisition boards were assembled in waterproof cases and powered by 50 W solar panels. The connection diagram of the Raspberry Pi-3, Labjack and solar panel used for the operation of the biosensor is presented in the supplementary information as Fig. S1.

Field test location

The water quality monitoring field test of the biosensor was carried out in Junction Creek, located in Greater Sudbury, Ontario, Canada. This stream is 25 km in length, with a mean depth of 0.5 m and has been impacted by atmospheric and liquid effluent discharges from area smelters and mine sites, road salt, channelization, urban storm, municipal sewage inputs, etc. (Weber et al. 2008). This discharge led to a decline in the water quality of the stream over several years until remediation efforts by several groups commenced around 1970 (Gunn et al. 2010). The ecotoxicity monitoring of the remediation

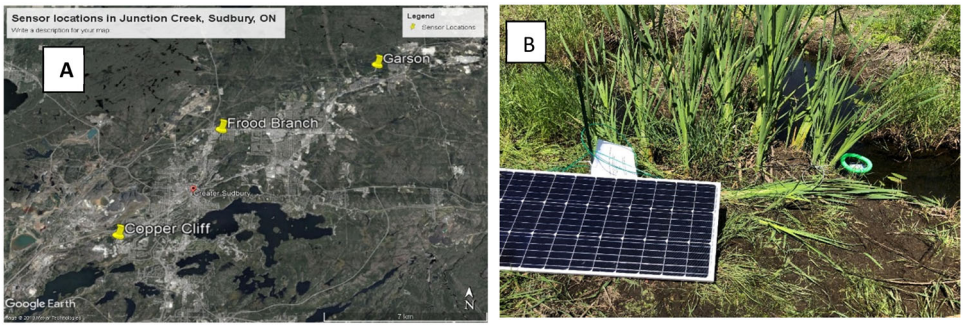

Fig. 2 Biosensor deployment locations in Junction Creek, Sudbury, ON (**A**), Biosensor deployment at Garson Branch (**B**)

efforts, however, is limited to periodic but non-continuous sampling campaigns by different groups, and also subject to the limitations of existing analytical methods. This stream therefore presents an opportunity to test the performance of an MFC based biosensor for water quality monitoring in real-time. Precipitation data for Sudbury, ON, Canada during the study was downloaded from the publicly available online database of Environment and Climate Change Canada.

Three sites within the Junction Creek stream were selected for testing the performance of the biosensor. These sites are namely Garson Branch (GPS 46.551083, −80.879315), Frood Branch (GPS 46.518368, −80.979470) and Copper Cliff Creek (GPS 46.47075,−81.05971). Figure 2A shows the biosensor deployment and water sampling locations and Fig. 2B is a picture of the deployment at Garson Branch. Briefly, a biosensor was installed in the Junction Creek headwaters near Garson mine before receiving any input from Garson wastewater treatment plant (WWTP) in the Garson branch. Another biosensor was installed at the Frood Branch before input from the Nolin Creek WWTP. Finally, a biosensor was installed after the effluent discharge zone of the Copper Cliff WWTP but upstream of the municipal sewage treatment plant (STP) effluent shortly before it emptied into Kelly Lake. Table 1 shows the heavy metals composition and concentration at the three sites during biosensors deployment.

Daphnia magna assay

During the biosensing field test, water samples were collected from the biosensors' deployment locations for simultaneous ecotoxicological testing. The assay was necessary so as to be able to compare real-time (biosensor) results with standardized (laboratory based) test results. In this work, the 48-h lethality test with *Daphnia magna* was chosen to access toxicity in the water samples due to its frequent use in metal mine effluent toxicity monitoring and its rapid testing time. The *D. magna* lethality tests were conducted in accordance with Environment and Climate Change Canada's biological test method (EPS1/RM/11). In brief, ten 24-h old *D. magna* were placed into 150 mL of MHW for 48 h. Experimental design consisted of 4 replicates per treatment and *D. magna* survival was assessed at 48 h. Weekly water samples collected at the biosensor deployment locations and sent overnight to the laboratory were used for the lethality tests. The water samples were not filtered prior to testing to ensure that *D. magna* were exposed to the same samples as the biosensor in the field. The relationship between metal concentrations (ug L^{-1} dissolved) in weekly water samples and *D. magna* survival (%) were assessed using a simple linear regression. Only those metals that were elevated to a concentration where an effect would likely be observed (e.g. greater than a reported EC50 value) were identified for regression analysis.

Microtox assay

After an analysis of the field test results, the Microtox assay (Bulich 1986) was carried out to investigate the ecotoxicological implications of the interactions between the two dominant heavy metals identified at the field test location. The Microtox assay is based on the luminescence of *Vibrio fischeri* exposed to water samples as specified in the Environmental Protection Series manual (Environment Canada C 1992). NaCl (0.4 M) was used to dilute the bacterial culture. Reconstitution solutions were supplied by Modern Water (Modern Water Inc, Newcastle, DE, USA). The luminescence at start (T_0), and after 15 min (T_{15}) were measured using a MicroBics M500 toxicity analyzer (Bio-Microbics Inc, KS, USA) and analyzed in Microsoft Excel 2017 (Microsoft Corporation, Redmond, WA, USA). Two tailed Student *t*-tests were carried out on all Microtox data, also in Microsoft Excel 2017.

Results and discussion

It is important to note that microbial fuel cell biosensors are not specific analyte detectors but rather give an indication or

Table 1 General water quality (pH, DOC (%)), conductivity (μs cm^{-1}) and concentrations of total and dissolved metals at Garson Branch during the field test

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
pH	7.09	7.20	7.15	7.18	7.24	7.14	7.27	7.14
DOC (%)	108.20	100.90	104.20	105.30	106.30	110.00	108.40	105.80
Conductivity (μs/cm)	2360.00	2400.00	1981.00	1980.67	2210.00	2340.00	2121.00	2210.00
TOTAL								
Al (μg L^{-1})	31.5 ± 1.8	28.6 ± 6.3	16.0 ± 3.6	13.5 ± 1.9	10.2 ± 0.8	8.5 ± 1.5	10.5 ± 2.1	20.1 ± 5.9
As (μg L^{-1})	2.4 ± 0.19	2.7 ± 0.2	2.4 ± 0.1	2.1 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.5 ± 0.2	1.4 ± 0.0
Ca (μg L^{-1})	283.3 ± 3.9	311.7 ± 07.9	263.8 ± 3.6	252.1 ± 0.7	274.2 ± 1.8	301.0 ± 6.1	267.3 ± 3.0	269.5 ± 5.8
Cd (μg L^{-1})	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Co (μg L^{-1})	11.9 ± 0.4	14.9 ± 0.9	11.7 ± 3.7	13.4 ± 3.7	8.0 ± 0.6	7.9 ± 1.2	7.7 ± 0.1	5.9 ± 0.1
Cu (μg L^{-1})	6.6 ± 0.2	6.2 ± 0.1	4.9 ± 0.1	4.7 ± 0.1	3.3 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	3.2 ± 0.2
Fe (μg L^{-1})	419.5 ± 9.9	460.6 ± 27.8	461.2 ± 55.5	333.8 ± 55.5	267.8 ± 17.9	270.6 ± 21.4	453.9 ± 6.1	376.2 ± 8
Mn (μg L^{-1})	352.0 ± 8.6	585.8 ± 16.2	543.6 ± 70.7	668.8 ± 70.7	710.8 ± 16.1	693.0 ± 43.4	310.3 ± 2.0	179.2 ± 3.6
Na (mg L^{-1})	127.9 ± 2.9	159.2 ± 2.8	139.6 ± 1.2	101.7 ± 1.2	109.9 ± 0.4	117.7 ± 2.7	102.1 ± 1.7	103.6 ± 3.4
Ni (μg L^{-1})	324.5 ± 1.4	341.4 ± 3.2	268.8 ± 4.9	363.2 ± 4.9	235.9 ± 1	245.7 ± 6.5	211.1 ± 1.7	219.6 ± 5.7
Pb (μg L^{-1})	4.8 ± 0.3	4.5 ± 0.1	3.7 ± 0.02	3.6 ± 0.0	3.3 ± 0.1	3.5 ± 0.5	3.3 ± 0.2	3.6 ± 0.4
Se (μg L^{-1})	1.0 ± 0.01	1.0 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.7 ± 0.0	0.7 ± 0.1	0.9 ± 0.1
DISSOLVED								
Al (μg L^{-1})	3.9 ± 0.3	4.5 ± 0.1	7.9 ± 0.4	6.0 ± 0.5	4.6 ± 0.2	4.2 ± 0.2	2.3 ± 0.1	2.2 ± 0.1
As (μg L^{-1})	2.2 ± 0.3	2.1 ± 0.3	1.8 ± 0.2	1.4 ± 0.3	1.3 ± 0.2	1.2 ± 0.3	1.1 ± 0.2	0.9 ± 0.3
Ca (μg L^{-1})	296.7 ± 2.5	325.2 ± 3.5	265.7 ± 2.7	254.8 ± 3.5	270.0 ± 3.9	298.8 ± 1.3	271.5 ± 6.2	278648.7 ± 4.8
Cd (μg L^{-1})	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.0	0.1 ± 0.01	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.01
Co (μg L^{-1})	5.7 ± 0.2	13.6 ± 0.1	12.4 ± 0.3	6.9 ± 0.03	4.7 ± 0.03	2.8 ± 0.04	7.6 ± 0.3	5.9 ± 0.02
Cu (μg L^{-1})	4.2 ± 0.1	4.3 ± 0.1	3.8 ± 0.1	4.1 ± 0.1	2.8 ± 0.04	2.5 ± 0.3	1.7 ± 0.1	2.1 ± 0.02
Fe (μg L^{-1})	17.4 ± 2.0	27.6 ± 6.5	77.8 ± 11.8	31.4 ± 3.8	13.6 ± 3.1	12.9 ± 0.7	13.7 ± 1.3	17.6 ± 1.3
Mn (μg L^{-1})	204.6 ± 5.7	581.9 ± 4.6	559.6 ± 4.1	581.6 ± 7.3	632.9 ± 7.5	557.3 ± 0.5	312.1 ± 5.1	180.9 ± 0.9
Na (mg L^{-1})	136.6 ± 0.30	169.3 ± 1.6	142.3 ± 1.6	103.2 ± 1.3	108.7 ± 1.4	116.5 ± 0.08	104.8 ± 1.6	106.4 ± 0.5
Ni (μg L^{-1})	321.5 ± 4.8	330.2 ± 1.9	271 .9 ± 3.4	360.5 ± 3.3	227.0 ± 3.5	241.3 ± 1.3	211.2 ± 6.5	218.4 ± 1.3
Pb (μg L^{-1})	4.7 ± 0.05	4.1 ± 0.1	3.4 ± 0.1	3.4 ± 0.1	3.2 ± 0.02	3.1 ± 0.03	3.1 ± 0.1	3.1 ± 0.1
Se (μg L^{-1})	1.0 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.01	0.7 ± 0.1	0.7 ± 0.04	0.6 ± 0.1	0.8 ± 0.1

Values represent means ± 1 standard deviation

progression of water quality as shown in several studies (Adekunle et al. 2019, 2020). In streams and many other aquatic environments, several background heavy metal concentrations that contribute to overall toxicity may be found. These heavy metals come from several sources including mining, urban run-offs, sewage treatment plants, and industrial waste treatment plants. In the case of Junction Creek, Ni and Cu are expected to be at higher than background levels in its water samples as Ni-Cu ores are being actively mined and processed in the Sudbury mining area. Therefore Ni and Cu can be expected to be the drivers of toxicity in the field test.

Biosensor response at Garson Branch

Based on the water quality data, the water in this area of the stream can be characterized as moderately hard to very hard and mostly neutral pH, similar to results obtained in a previous study (Houle et al. 2007). It is also important to note that water quality parameters such as pH and dissolved organic matter influence heavy metal bioavailability, and accumulation in living organisms. Notably, the soluble Cu and Ni concentration in water samples at this location ranged between 2–4 μg L^{-1} and 211–360 μg L^{-1} respectively throughout the duration of the test (Table 1). Therefore, based on the water quality parameters obtained at this site, the CCME based site specific water quality guidelines, as well as our previous study (Adekunle et al. 2020), little to no Cu and Ni related toxicity can be expected at this location.

The biosensor output (V_{min}) at this location was the highest amongst all the three sites tested during this study. During the first three weeks of deployment, the biosensor signal increased briefly after each precipitation event (for example day 10 and 21) and decreased during dry periods as shown in Fig. 3A. With such isolated precipitation events, a decrease in the overall concentration of heavy metals and an increase in the dissolved organic matter (DOM) can be expected. In the following weeks, from around day 24, the biosensor signal was fairly stable with smaller daily fluctuations (days 25–38). This is explained by the reduction in Cu and Ni concentration after the

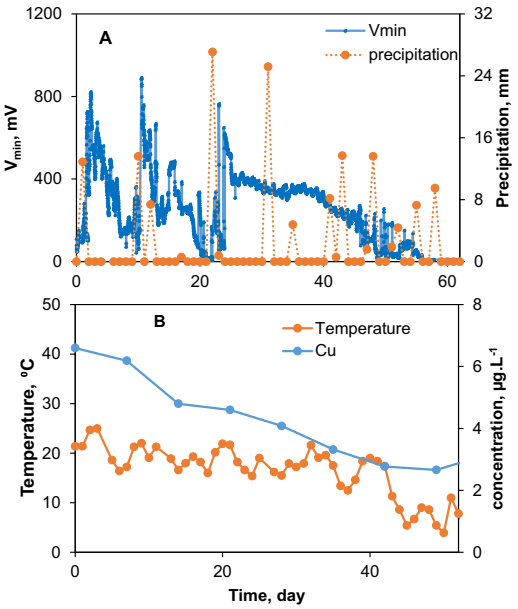

Fig. 3 Biosensor output (V_{min}) response (**A**), temperature, and Cu profile at Garson Branch (**B**)

deployment of the biosensor (Fig. 3B). In addition, it can be suggested that the microbial populations of the biosensor became more adapted after the initial 3 weeks, thereby reducing the sensitivity of the biosensor. In summary, the total metal concentrations at this location during the entire field test was low when compared to the other two sites (Tables 2 and 3).

Towards the end of the test (after day 40), the decline in the biosensor's output can be explained by several factors. First, an increase in the frequency of precipitation events leads to an increased flow rate of water in the stream, higher dissolved oxygen concentration and, accordingly, increased exposure of the anodic biofilm to dissolved oxygen. Also, a decrease in the carbon source supply (saw dust placed under the anode) in the MFC due to biodegradation, as shown in the previous work (Adekunle et al. 2017) can be hypothesized. Finally, due to the increased precipitation, despite bi-weekly maintenance, there was significant sludge accumulation on the air-breathing cathode, which potentially hampered oxygen availability for the oxygen reduction reaction at the cathode. Moreover, oxidation of the electrode connections and wires could be clearly seen at the end of the test. Nevertheless, the biosensor output recovered to 400 mV once it was cleaned up and returned to a water sample obtained from the same site in the laboratory (results not shown). The effect of a decline in temperature was observed to be minimal, even though temperature has been shown to affect the performance of MFCs in general (Jadhav and Ghangrekar 2009).

Biosensor response at Frood Branch

Historically, the water quality data at Frood Branch shows that it had a lower pH (4.9), an average Cu concentration of 88.04 µg L^{-1}, and an average Ni concentration of 715 µg L^{-1} (Gunn et al. 2010; Johnson and Owen 1966). Frood received acid mine drainage (AMD) from a waste rock pile until 2001 when the AMD was diverted for treatment in the Copper Cliff wastewater treatment plant. Due to remediation efforts, at the time of biosensor deployment, the pH was mostly neutral, although Cu and Ni concentration were higher than at Garson due to the input from the Garson WWTP and release from sediments in the area (Houle et al. 2007). In addition, the water hardness is lower when compared to the Garson site. In summary, the range of dissolved metals concentration at this location was 10–20 µg L^{-1}, and 40–70 µg L^{-1} for Cu and Ni, respectively.

After the biosensor deployment, there was a significant periodic variation in the biosensor output, in particular between days 7–9 (Fig. 4). The biosensor was able to recover several times, but eventually the biosensor output was reversed (−45 mV). Due to the configuration of the data acquisition system for only positive voltage measurements, data from Day 10–31 when the biosensor had a reversed voltage was not recorded. Voltage reversal generally occurs in MFCs when there is a deficiency in dissolved organic matter concentration, or metal toxicity is significant, as demonstrated in previous studies (Adekunle et al. 2019; Oh and Logan 2007). The observed behavior can be attributed to the periodic release of heavy metals from the sediment in this location as proven by several other previous studies (McGregor et al. 1998a, b; Nriagu et al. 1998; Stokes and Szokalo 1977). This periodic release is well reflected by the fluctuations in the biosensor's output. To demonstrate that the biosensor's response at this location in the initial 10 days after deployment was due to the effect of re-release of heavy metals, the biosensor was retrieved after 31 days, and tested in the laboratory. The biosensor was placed in moderately hard water and the output signal recovered to 85 mV immediately. The biosensor was subsequently maintained in the water collected at Garson Branch (no toxicity) for several days and reinstalled at the same location on Day 42 to restart water quality monitoring. Figure 4 inset shows the biosensor response and recovery in water samples from Garson Branch before a return to Frood Branch.

It can be seen from Fig. 4 that upon re-deployment there was an immediate decrease in the biosensor's output. This response shows clearly the difference between Garson Branch and Frood Branch in toxicity terms. Nevertheless, the biosensor can be seen to show relatively stable performance after the redeployment, for the remainder of the entire test. Notably, at this site, the Cu concentration at the later end of the test was stable and not fluctuating as much as can be observed at Garson Branch. Unlike the deployment location at Garson, the

Table 2 General water quality (pH, DO (%)), conductivity (μs cm^{-1}) and concentrations of total and dissolved metals at Frood Branch during the field test

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
pH	7.92	7.62	8.14	7.79	7.90	7.61	7.72	7.70
DO (%)	109.60	99.70	105.20	107.30	106.60	107.30	108.00	108.70
Conductivity (μs/cm)	1034.00	1160.00	754.00	776.00	1050.00	927.00	1076.00	1089.00
TOTAL								
Al (μg L^{-1})	74.1 ± 1.3	59.4 ± 1.5	86.1 ± 14.5	95.5 ± 35.2	31.2 ± 6.4	34.7 ± 5.4	63.2 ± 6.3	43.3 ± 0.7
As (μg L^{-1})	2.6 ± 0.2	1.7 ± 0.2	1.5 ± 0.2	1.1 ± 0.1	1.2 ± 0.1	1.0 ± 0.2	1.2 ± 0.1	1.0 ± 0.2
Ca (μg L^{-1})	41.7 ± 0.1	53.4 ± 0.4	37.7 ± 0.7	35.7 ± 2.4	46.6 ± 0.7	45.8 ± 0.6	51.3 ± 1.4	50.7 ± 1.3
Cd (μg L^{-1})	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.03	0.1 ± 0.0	0.3 ± 0.4	0.1 ± 0.01	0.1 ± 0.0
Co (μg L^{-1})	1.0 ± 0.02	0.9 ± 0.03	0.8 ± 0.1	0.9 ± 0.3	0.5 ± 0.1	0.4 ± 0.04	0.7 ± 0.04	0.4 ± 0.04
Cu (μg L^{-1})	20.5 ± 0.58	23.3 ± 0.4	31.1 ± 1.4	23.4 ± 1.4	17.0 ± 0.2	13.2 ± 0.5	16.4 ± 0.2	15.2 ± 0.2
Fe (μg L^{-1})	910.9 ± 0.2	806.2 ± 0.2	661.7 ± 0.9	454.3 ± 0.1	465.1 ± 0.5	327.0 ± 0.3	482.2 ± 3.1	376.0 ± 3.5
Mn (μg L^{-1})	95.6 ± 2.0	78.8 ± 1.4	40.0 ± 5.5	51.8 ± 20.9	33.1 ± 4.3	36.6 ± 3.8	51.8 ± 2.1	30.0 ± 2.0
Na (mg L^{-1})	147.1 ± 3.0	181.3 ± 4.1	125.2 ± 3.8	83.4 ± 1.9	117.7 ± 0.3	97.3 ± 1.4	113.7 ± 1.1	115.5 ± 0.9
Ni (μg L^{-1})	64.6 ± 0.1	74.9 ± 0.3	79.4 ± 2.2	74.8 ± 5.7	51.9 ± 1.3	46.4 ± 1.0	68.2 ± 0.6	70.1 ± 1.6
Pb (μg L^{-1})	4.4 ± 0.04	4.0 ± 0.04	3.6 ± 0.4	3.4 ± 0.2	3.4 ± 0.1	3.1 ± 0.1	3.2 ± 0.1	3.2 ± 0.04
Se (μg L^{-1})	0.9 ± 0.13	1.0 ± 0.1	1.2 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
DISSOLVED								
Al (μg L^{-1})	13.9 ± 0.1	7.1 ± 0.1	28.7 ± 1.8	18.3 ± 0.5	16.1 ± 8.0	6.9 ± 0.2	5.9 ± 0.2	4.2 ± 0.1
As (μg L^{-1})	1.7 ± 0.1	1.5 ± 0.2	1.1 ± 0.3	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
Ca (μg L^{-1})	42.4 ± 0.05	53.7 ± 0.2	37.5 ± 0.2	35.8 ± 0.2	46.2 ± 0.3	45.8 ± 0.1	51.9 ± 0.6	51.9 ± 0.6
Cd (μg L^{-1})	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Co (μg L^{-1})	0.3 ± 0.01	0.3 ± 0.01	0.4 ± 0.01	0.3 ± 0.02	0.3 ± 0.01	0.2 ± 0.01	0.4 ± 0.01	0.2 ± 0.02
Cu (μg L^{-1})	13.4 ± 0.2	16.0 ± 0.3	20.5 ± 0.3	17.4 ± 0.04	12.6 ± 0.2	10.6 ± 0.2	11.5 ± 0.3	11.6 ± 0.3
Fe (μg L^{-1})	18.4 ± 2.6	15.9 ± 3.9	74.2 ± 0.1	40.5 ± 3.5	15.8 ± 3.7	8.0 ± 0.8	11.1 ± 4.6	8.7 ± 0.9
Mn (μg L^{-1})	11.3 ± 0.6	17.0 ± 0.1	12.8 ± 1.3	10.0 ± 1.0	8.1 ± 0.2	9.1 ± 0.3	30.5 ± 0.8	11.6 ± 0.4
Na (mg L^{-1})	151.8 ± 0.8	182.5 ± 1.0	123.4 ± 0.7	82.6 ± 0.9	116.3 ± 1.5	95.8 ± 0.3	115.1 ± 0.4	119.1 ± 2.5
Ni (μg L^{-1})	54.2 ± 0.4	68.4 ± 0.6	66.3 ± 0.4	64.6 ± 0.6	44.3 ± 0.7	41.9 ± 0.6	71.5 ± 12.1	69.2 ± 0.8
Pb (μg L^{-1})	4.1 ± 0.2	3.9 ± 0.04	3.0 ± 0.03	2.9 ± 0.04	30.5 ± 23.8	2.8 ± 0.01	3.0 ± 0.1	3.2 ± 0.4
Se (μg L^{-1})	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.04	0.9 ± 0.2

Values represent means ± 1 standard deviation

Frood Branch deployment location is filled with a lot of branches which reduced the flow of water even during increased precipitation. In addition, the effect of cleaning and replacing the electrode connections may have also contributed to the stability in the biosensor output noted after its re-deployment.

Biosensor response at Copper Cliff

The Copper Cliff WWTP in Sudbury treats water from active tailings, inactive tailings, surface drainage, active mining, milling, refining and smelting from the Sudbury mining complex and discharges effluents into Junction Creek at the Copper Cliff Creek site. As expected, the Copper Cliff WWTP inputs the highest volume of mining related waste into Junction Creek at the Copper Cliff Creek location (Weber et al. 2008). It has the highest pH average (8.2) of all the sites in this study, due to the liming process that occurs during the mining wastewater treatment process. Dissolved Cu concentrations ranged between 10–30 μg L^{-1} while Ni concentration ranged between 600–5000 μg L^{-1} (Table 3).

Two biosensors were deployed at this location during this study to confirm biosensor performance in a relatively high toxicity area. Immediately after deployment, a progressive and rapid but steady decrease in the biosensors output can be observed in both biosensors (Fig. 5). This result is similar to that obtained in our previous study (Adekunle et al. 2020), where this location was monitored for about 10 days using one biosensor. The results of this study therefore confirm that observations from the previous test are valid since this location had the highest concentration of metals and range change during the test. For example, Cu concentration increased up to approximately 80 μg L^{-1} within the first seven days of water quality monitoring. Unfortunately, the uncontrolled conditions within the stream, such as high flow rates and debris accumulation led to a mechanical failure (wire break) of the data collection setup, even though the biosensor remained in the water. As may be expected due to the creek's conditions, the biosensors outputs would have declined rapidly. By Day 32, a manual measurement of the biosensor voltage showed that the outputs of both biosensors had reversed to approximately −150 mV. The reversed voltage was higher

Table 3 General water quality (pH, DO (%)), conductivity (μs cm^{-1}) and concentrations of total and dissolved metals at Copper Cliff during the field test

	Week 1	Week 2	Week 3	Week 4
pH	7.70	7.78	7.68	7.55
DO (%)	112.60	100.40	104.60	104.10
Conductivity (μs/cm)	1332.00	1182.00	844.00	2250.00
TOTAL				
Al (μg L^{-1})	77.8 ± 2.9	55.3 ± 1.9	80.1 ± 2.7	16.7 ± 0.3
As (μg L^{-1})	1.7 ± 0.2	2.3 ± 0.1	2.0 ± 0.1	1.2 ± 0.1
Ca (μg L^{-1})	113.3 ± 1.7	70.2 ± 0.5	48.2 ± 1.6	338.3 ± 3.8
Cd (μg L^{-1})	1.2 ± 0.02	1.5 ± 0.02	1.9 ± 0.05	0.4 ± 0.02
Co (μg L^{-1})	57.2 ± 0.9	81.4 ± 0.7	103.5 ± 2.5	11.6 ± 0.03
Cu (μg L^{-1})	32.2 ± 0.6	34.5 ± 0.3	79.1 ± 2.7	13.5 ± 0.1
Fe (μg L^{-1})	629.2 ± 13.5	710.5 ± 7.8	959.5 ± 22.3	212.6 ± 1.0
Mn (μg L^{-1})	157.1 ± 3.5	151.5 ± 1.6	169.0 ± 5.1	33.6 ± 0.8
Na (mg L^{-1})	142.6 ± 2.1	171.4 ± 0.7	127.5 ± 3.7	126.9 ± 0.2
Ni (μg L^{-1})	3184.3 ± 55.3	4638.0 ± 58.6	5667.7 ± 0.2	636.0 ± 5.2
Pb (μg L^{-1})	4.2 ± 0.02	3.8 ± 0.1	3.5 ± 0.1	3.0 ± 0.1
Se (μg L^{-1})	6.2 ± 0.2	6.3 ± 0.3	6.0 ± 0.2	16.3 ± 0.4
DISSOLVED				
Al (μg L^{-1})	7.6 ± 0.04	7.2 ± 0.3	11.2 ± 1.0	9.2 ± 1.3
As (μg L^{-1})	1.2 ± 0.2	1.4 ± 0.2	1.2 ± 0.1	1.2 ± 0.2
Ca (μg L^{-1})	116.8 ± 0.9	70.8 ± 1.2	41.9 ± 0.6	337.9 ± 3.8
Cd (μg L^{-1})	1.0 ± 0.1	1.3 ± 0.04	1.6 ± 0.02	0.3 ± 0.02
Co (μg L^{-1})	55.8 ± 0.4	80.1 ± 0.1	83.7 ± 1.6	10.9 ± 0.1
Cu (μg L^{-1})	14.3 ± 0.3	16.2 ± 0.2	30.9 ± 1.2	9.0 ± 0.1
Fe (μg L^{-1})	9.5 ± 1.9	13.5 ± 4.2	81.3 ± 19.2	16.7 ± 2.7
Mn (μg L^{-1})	143.1 ± 1.9	144.6 ± 2.3	140.9 ± 2.1	27.5 ± 0.9
Na (mg L^{-1})	148.9 ± 0.9	172.6 ± 1.9	84.9 ± 1.5	127.5 ± 1.3
Ni (μg L^{-1})	3201.0 ± 19.5	4633.0 ± 55.7	4897.0 ± 68.8	631.0 ± 7.4
Pb (μg L^{-1})	3.9 ± 0.1	3.7 ± 0.04	3.0 ± 0.2	2.7 ± 0.1
Se (μg L^{-1})	6.4 ± 0.2	6.2 ± 0.2	5.5 ± 0.4	16.4 ± 0.1

Values represent means ± 1 standard deviation

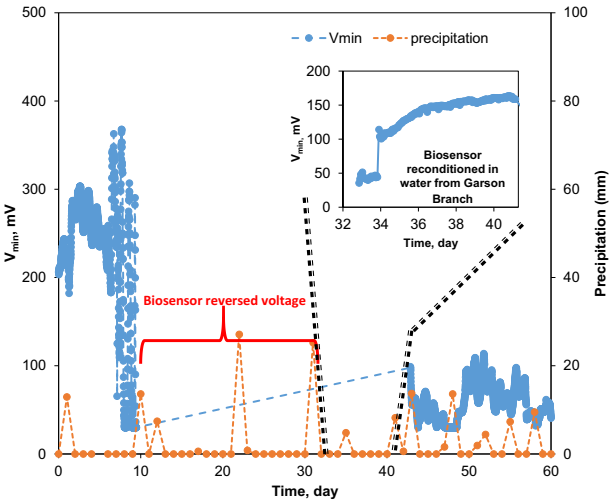

Fig. 4 Biosensor response at Frood Branch. Reversed voltage between day 10 and 31 and during the re-conditioning not captured. Inset: Response of the same biosensor when reconditioned in a water sample from Garson Branch before return to Frood Branch

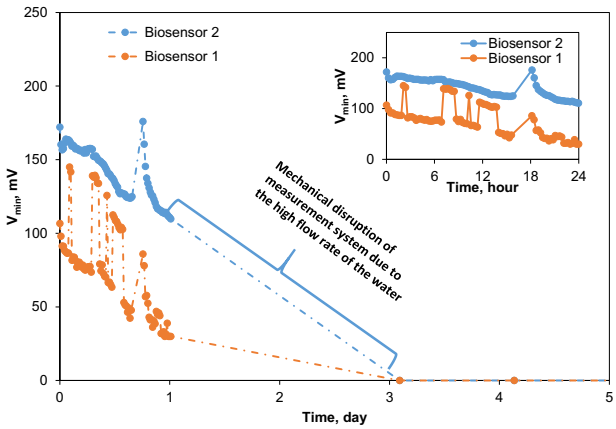

Fig. 5 Biosensor response at Copper Cliff. Data acquisition failed due to mechanical problem caused by high water current and debris accumulation. Inset: Biosensor response over the first 24 h of deployment

than was measured in the first week at Frood Branch (−45 mV), confirming that toxicity was more pronounced at Copper Cliff.

Daphnia assay toxicity monitoring and comparison with biosensor responses

A standardized toxicity test (the *Daphnia magna* lethality assay) was used to determine toxicity in aquatic environments. This test was carried out simultaneously with the field tests to evaluate toxicity and compare it to the biosensor response. Figure 6 illustrates toxicity observed over the 8 weeks during which the biosensors were also deployed at each site. Very little toxicity was observed over the 8 week testing period for both Garson Branch and Frood Branch sites. Copper Cliff, however, did demonstrate substantial toxicity in the second and third week of sampling, which agrees with the biosensor outputs (i.e. the biosensors installed at Copper Cliff showed significant toxicity leading to voltage reversal). When concentrations of metals are compared to reported EC50 values for *D. magna*, only Cu and Ni are identified as having concentrations that could exhibit a toxic response (Okamoto et al. 2014). The survival of *D. magna* compared to concentrations of Cu and Ni was therefore assessed using a linear regression. Copper corresponded well with lethality at Copper Cliff ($R_2 =$ 0.95) compared to Ni ($R_2 = 0.53$) suggesting that Cu was the primary driver of toxicity at this site (Table 3). However, it is important to note that the nature of metal mixtures (i.e. additive, antagonistic or synergistic relationships) were not accounted for in this analysis. Considering the protective response of Ni observed in the Microtox tests (section 3.5) this should be investigated further. The lack of effects at Frood Branch suggests that the biosensor is more sensitive to changes in water quality compared to *D. magna*. However, it should be highlighted that the *D. magna* lethality test is an

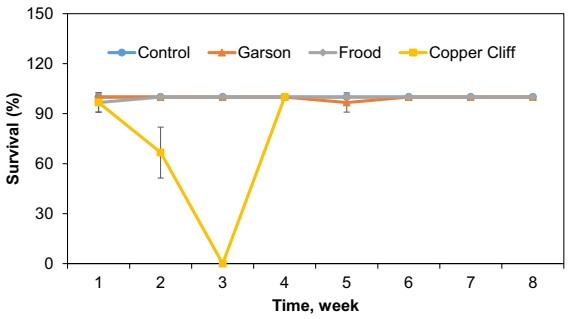

Fig. 6 Survival (%) of *D. magna* after 48 h exposure to unfiltered site water collected weekly during deployment of the remote biosensor in Sudbury, ON

acute response assessment based on one-grab sample. The biosensor, on the other hand, may have been exposed to the release cycles of heavy metals from the sediment in the first few days, leading to an eventual chronic response. Evaluating chronic toxicity at this location, while comparing it with the biosensing tests, is recommended for future studies.

The ecotoxicological contributions of Cu and Ni in Junction Creek

The detection of Cu toxicity by MFC based biosensors have been shown in several studies (Adekunle et al. 2020; Zhu and Logan 2014). On the other hand, Ni has been shown to be a useful catalyst in improving the cathodic half-reaction in MFCs (Huang et al. 2015) although toxicity at higher (mg L^{-1}) concentrations have also been shown (Stein et al. 2012). It is therefore important to investigate the contributions of these two dominant heavy metals to the toxicity profile recorded by the biosensor and the *D. magna* assay.

The Microtox assay was used to investigate the interaction of Ni and Cu by examining the bioluminescence of *Vibro fisheri* to MHW, Cu, Ni solutions as well as a 1:3 and 1:10 mix of Cu and Ni respectively. Figure 7A shows the different effects of the heavy metals presence, type, and concentration on the luminescence of *V. fischeri* after 15 min (T_{15}) compared to the start of the experiment (T_0). A summary of a Student's *t*-test analysis carried out on the Microtox data is also shown on Table 4. It is clear that the presence of Cu at concentrations as low as 20 μg L^{-1} decreases bioluminescence at a statistically significant level ($t = 6.58$, $p = .001$). In contrast, the presence of Ni up to 600 μg L^{-1} has no statistically significant negative effect on the bioluminescent bacteria ($t = 1.45$, $p = 0.21$). In fact, a slight improvement in the bioluminescence can be seen at 600 μg L^{-1} of Ni. As discovered by Rodrigue et al. (2005), there is an inherent nickel defense system (RcnR/RcnA) found in microorganisms that live in a wide range of environmental niches. Also, Ni toxicity has been found to be pH dependent in several studies (Babich and Stotzky 1982). These pH dependent toxicity and microbial homeostasis can be suggested as the reason for the response of *V. fisheri* in the Microtox assay. Interestingly, it can also be hypothesized from the Microtox results that the combined effect of both Cu and Ni depends on the ratio and concentration of both metals. Clearly, the progressive, protective effect of Ni up until 600 μg L^{-1} can be seen in the Microtox assay. The reduction in the toxic response (decrease in bioluminescence) is apparent in Fig. 7A when the response to the Cu: Ni (20:200 μg L^{-1}) mixture is compared to Cu 20 μg L^{-1} solution. Furthermore, the Cu: Ni (200:600 μg L^{-1}) mixture of Cu and Ni was not toxic, with no significant difference between T0 and T15 ($t = 0.19$, $p = .85$). A similar antagonistic effect between Cu and Ni was identified in earlier studies at higher concentrations (>1 mg L^{-1}) and acidic pH (Al-Hejuje 2012; Babich and Stotzky 1983).

While it is important to highlight the limited exposure time of the Microtox assay, the observation from the Microtox assay, however, validates the toxicity monitoring performance of the biosensor at all locations, where Cu and Ni are major constituents (Tables 1–3). At Garson Branch, Cu concentration was ≤20 μg L^{-1} while Ni concentration was ≤600 μg L^{-1} throughout the duration of the test. According to the Microtox data, a low toxicity situation is expected at these concentrations, explaining the relatively higher biosensor signal (when compared to the two other sites) throughout the duration of the test. During the first two weeks of deployment, at Frood Branch, Cu concentration was ≥20 μg L^{-1} while Ni concentration was ≤80 μg L^{-1}. According to the interpretation of the Microtox data, due to a reduced protective effect of Ni, a poorer water quality at Frood Branch (compared to Garson Branch) can be implied. Therefore, the biosensor signal trend and subsequent reversal by day 10 can be closely related to the changing water quality at Frood Branch. Furthermore, at the redeployment of the biosensor on day 42, Cu and Ni concentration at Frood Branch was approximately 13 and 46 μg L^{-1} respectively. With an improved water quality due to a lower Cu concentration, the stable sensor performance for the remainder of the test is unsurprising. At Copper Cliff, with Ni concentration higher than 3000 μg L^{-1} at deployment, the rapid decline and subsequent reversal of the biosensor signal follows a trend that can be expected from the interpretation of the Microtox test. Altogether, the biosensor's overall water quality monitoring performance can be described as satisfactory with respect to the Microtox assay, even without considering the temporal water quality data advantage of the biosensor.

The *D. magna* lethality observed at the Copper Cliff watershed corresponded well with Cu and Ni concentrations measured in the site water. To compare the response of *D. magna* in the field water tests with Cu and Ni, an assessment of both metals was conducted using organisms from the same culture and test materials to determine LC_{50s}. The methods followed exactly that of the field tests (Section

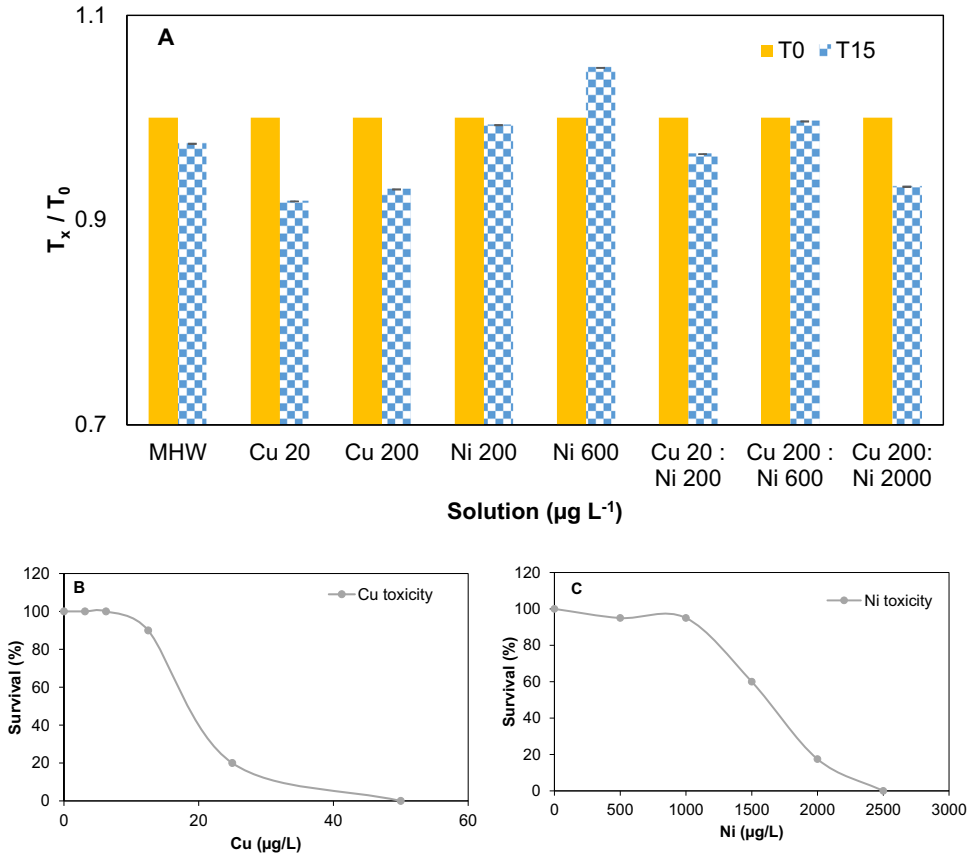

Fig. 7 **A** Bioluminescence of V. fisheri (Tx/To) as a function of Cu and Ni concentration in a Microtox assay and (**B**, **C**) Lethal toxicity of Cu and Ni to D. magna. Standard deviations for Fig. 7A are shown as error bars

Table 4 Summary of Student's *t* tests on microtox data

Solution type	Microtox assay data				
		T0	T15	*t* value	prob
MHW	M SD	91 (1.63)	88 (1.69)	1.27	0.30
Copper	M SD	92 (1.91)	85 (1.83)	6.58	0.001
Nickel	M SD	89.8 (1.07)	91.7 (2.75)	1.45	0.21
Cu: Ni mixtures (20:200)	M SD	84 (0.00)	81 (1.41)	3.00	0.03
Cu: Ni mixtures (200: 600)	M SD	88.7 (1.25)	88.7 (2.05)	0.14	0.89
Cu: Ni mixtures (200: 2000)	M SD	93.7 (2.49)	87.3 (0.94)	3.36	0.02

2.4) using a dilution series of $CuSO_4$ and NiCl. The results are illustrated in Fig. 7B, C. LC_{50s} values of 24.4 µg L^{-1} and 1505 µg L^{-1} were calculated for Cu and Ni, respectively. When concentrations at the various sites along Junction Creek are compared to these LC_{50s}, the lack of toxicity at Garson Branch can be explained, as concentrations of both Cu and Ni are well below the LC_{50s} values. Similarly, at Frood Branch the lack of toxicity can be explained by low Cu and Ni concentrations with the exception of week 3, which saw a Cu concentration of 31 µg L^{-1}. Interestingly, Cu and Ni at Copper Cliff were significantly greater, with the highest concentrations observed in weeks 2 and 3. The lack of toxicity at week 1 and 4 despite elevated Cu and Ni concentrations could be due to protective effect of hardness. Increased hardness reduces Cu and Ni toxicity in freshwater and hardness concentrations at all three sites was greater than 400 mg L^{-1} $CaCO_3$ (Tables 1–3) throughout the testing period. These values correspond to very hard water. Comparatively, the test water during the Cu and Ni toxicity experiments was categorized as moderately hard and therefore less protective. Overall, the LC_{50} values compare well with the field water characterization, where Cu and Ni concentrations exceeded 30 µg L^{-1} and 4000 µg L^{-1}, respectively. When the toxicity was compared with the biosensor response, it was observed that the biosensor output was comparable to *D. magna* toxicity. For example, the biosensor output (V_{min}) at Garson was the highest amongst all the three sites and lowest at Copper Cliff which corresponds well with our toxicity testing where no lethality was observed at Garson and acute lethality was observed at Copper Cliff. However, the biosensor did show increased sensitivity to Junction Creek water, for example at Frood branch where

changes in output in the biosensor signal were identified, but no comparable toxicity was observed. The difference in sensitivities could be due to species differences (i.e. microbial versus invertebrate responses) but it is most likely due to the response variable, a chronic response with the biosensor versus an acute response with *D. magna*. The inclusion of a chronic toxicity test would provide a more comparable response variable to the biosensor and should be considered for future testing.

In addition, one aspect that was not investigated with *D. magna* was the mixture of metals (particularly Cu and Ni) as in the Microtox assay. As with any field water assessment, multi-metal exposure should be taken into account. Therefore the mixture of Cu and Ni will be further investigated to compare with the single metal exposures, the field biosensor response and the Microtox assay results.

Conclusion

This study evaluated field performance of MFC-based biosensors for on-line monitoring of heavy metals related toxicity. Importantly, the biosensors showed an acceptable correlation with the toxicity profiles observed at three different locations corresponding to different Cu and Ni concentrations. The contributions of the Cu and Ni to the toxicity profiles at these sites show that Cu is the main toxicant, especially at neutral pH. Finally, it was shown that the results of an MFC based biosensor correlates with the results from the *D. magna* assay carried out at regular intervals simultaneously with the field test. The biosensor provides continuous toxicity monitoring in real time thus providing a better overview of toxicity as compared to a single grab sample approach of traditional methods. Overall, this study for the first time provides field results of real-time water quality monitoring using several MFC-based biosensors. These results can be used to improve the biosensor design to ensure its long-term stability in a field. In particular, design modifications leading to reduced debris accumulation, waterproof wire connections and improved connectivity, e.g. through a wireless communication system, are some of the improvements planned for subsequent tests.

Data availability

Raw data is available on request.

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Author contributions All the authors participated in research conceptualization, methodology, investigation, data curation and analysis and paper preparation.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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References

Adekunle A, Raghavan V, Tartakovsky B (2017) Carbon source and energy harvesting optimization in solid anolyte microbial fuel cells. J Power Sources 356:324–330. https://doi.org/10.1016/j.jpowsour.2017.01.062

Adekunle A, Raghavan V, Tartakovsky B (2019) On-line monitoring of heavy metals-related toxicity with a microbial fuel cell biosensor. Biosens Bioelectron 132:382–390. https://doi.org/10.1016/j.bios.2019.03.011

Adekunle A, Rickwood C, Tartakovsky B (2020) Online monitoring of heavy metal–related toxicity using flow-through and floating microbial fuel cell biosensors. Environ Monit Assess 192:52

Al-Hejuje M (2012) A synergistic effect of copper and nickel ions on the growth rates of Pseudomonas aeroginosa and Staphylococcus aureus isolates. Basrah J Sci(B) 30:78–93

Babich H, Stotzky G (1982) Nickel toxicity to microbes: effect of pH and implications for acid rain. Environ Res 29:335–350

Babich H, Stotzky G (1983) Synergism between nickel and copper in their toxicity to microbes: mediation by pH. Ecotoxicol Environ Saf 7:576–587. https://doi.org/10.1016/0147-6513(83)90017-9

Beauchemin D (2010) Inductively coupled plasma mass spectrometry. Anal chem 82:4786–4810

Besley CHChessman BCJEI (2008) Rapid biological assessment charts the recovery of stream macroinvertebrate assemblages after sewage discharges cease 8:625–638

Brezonik P, King S, Mach CJIMEC, (1991) Applications. Advances in Trace Substances Research Series. The influence of water chemistry on trace metal bioavailability and toxicity to aquatic organisms. Lewis Publishers I, Chelsea, Michigan. p 1–31. 10 fig, 5 tab, 82 ref

Bulich AA (1986) Bioluminescence assays. Toxicity testing using microorganisms 1:57–74

Debabov VG (2008) Electricity from microorganisms. Microbiology 77:123–131. https://doi.org/10.1134/S002626170802001X

Environment Canada C (1992) Biological test method: toxicity test using luminescent bacteria (Photobacterium phosphoreum)

Erickson RJ, Benoit DA, Mattson VR, Leonard EN, Nelson HP (1996) The effects of water chemistry on the toxicity of copper to fathead minnows. Environ Toxicol Chemistry 15:181–193. https://doi.org/10.1002/etc.5620150217

Gunn J, Sarrazin-Delay C, Wesolek B, Stasko A, Szkokan-Emilson EJH, Assessment ER(2010) Delayed recovery of benthic macroinvertebrate communities in Junction Creek, Sudbury, Ontario, after the diversion of acid mine drainage 16:901–912

Herlory O, Bonzom J-M, Gilbin R, Frelon S, Fayolle S, Delmas F, Coste M (2013) Use of diatom assemblages as biomonitor of the impact of treated uranium mining effluent discharge on a stream: case study of the Ritord watershed (Center-West France). Ecotoxicology 22:1186–1199. https://doi.org/10.1007/s10646-013-1106-5

Houle J, Oman K, Gunn J (2007) Water Quality and Thermal Conditions in Upper Junction Creek and its Tributaries. Junction Creek Restoration Steering Committee. *Unpublished*

Huang J, Zhu N, Yang T, Zhang T, Wu P, Dang Z (2015) Nickel oxide and carbon nanotube composite (NiO/CNT) as a novel cathode

non-precious metal catalyst in microbial fuel cells. Biosens Bioelectron 72:332–339

Hyne RV, Pablo F, Julli M, Markich SJJET, Journal CAI (2005) Influence of water chemistry on the acute toxicity of copper and zinc to the cladoceran. Ceriodaphnia cf dubia 24:1667–1675

Jadhav GS, Ghangrekar MM (2009) Performance of microbial fuel cell subjected to variation in pH, temperature, external load and substrate concentration. Bioresour Technol 100:717–723. https://doi.org/10.1016/j.biortech.2008.07.041

Jiang Y, Liang P, Huang X, Ren ZJ (2018) A novel microbial fuel cell sensor with a gas diffusion biocathode sensing element for water and air quality monitoring. Chemosphere 203:21–25. https://doi.org/10.1016/j.chemosphere.2018.03.169

Jiang Y, Liang P, Zhang C, Bian Y, Yang X, Huang X, Girguis PR (2015) Enhancing the response of microbial fuel cell based toxicity sensors to Cu(II) with the applying of flow-through electrodes and controlled anode potentials. Bioresour Technol 190:367–372. https://doi.org/10.1016/j.biortech.2015.04.127

Johnson M, Owen G (1966) Biological survey of the streams and lakes of the Sudbury area: 1965

Kim BH, Chang IS, Gadd G (2010) Microbial fuel cells as biochemical oxygen demand (BOD) and toxicity sensors. Bioelectrochemical systems. IWA Publishing, pp 347-367

McGregor R, Blowes D, Jambor J, Robertson WJEG (1998a) Mobilization and attenuation of heavy metals within a nickel mine tailings impoundment near Sudbury, Ontario. Canada 36:305–319

McGregor R, Blowes D, Jambor J, Robertson WJJoCH (1998b) The solid-phase controls on the mobility of heavy metals at the Copper Cliff tailings area, Sudbury, Ontario. Canada 33:247–271

Nriagu JO, Wong HK, Lawson G, Daniel PJSotTE (1998) Saturation of ecosystems with toxic metals in Sudbury basin, Ontario, Canada 223:99-117

Oh SE, Logan BE (2007) Voltage reversal during microbial fuel cell stack operation. J Power Sources 167:11–17. https://doi.org/10.1016/j.jpowsour.2007.02.016

Okamoto A, Yamamuro M, Tatarazako N (2014) Acute toxicity of 50 metals to *Daphnia magna*. J Appl Toxicol 35:824–830

Rodrigue A, Effantin G, Mandrand-Berthelot M-A (2005) Identification of rcnA (yohM), a Nickel and Cobalt Resistance Gene in Escherichia coli. J Bacteriol 187:2912–2916. https://doi.org/10.1128/jb.187.8.2912-2916.2005

Stein NE, Hamelers HMV, Van Straten G, Keesman KJ (2012) On-line detection of toxic components using a microbial fuel cell-based biosensor. J Process Control 22:1755–1761. https://doi.org/10.1016/j.jprocont.2012.07.009

Stein NE, Keesman KJ, Hamelers HVM, van Straten G (2011) Kinetic models for detection of toxicity in a microbial fuel cell based biosensor. Biosens Bioelectron 26:3115–3120. https://doi.org/10.1016/j.bios.2010.11.049

Stokes P, Szokalo AJWQRJ (1977) Sediment-water interchange of copper and nickel in experimental aquaria 12:157–178

Sun J-Z et al. (2015) Microbial fuel cell-based biosensors for environmental monitoring: a review 71:801–809

Weber LP, Dubé MG, Rickwood CJ, Driedger K, Portt C, Brereton C, Janz DM (2008) Effects of multiple effluents on resident fish from Junction Creek, Sudbury, Ontario. Ecotoxicol Environ Saf 70:433–445. https://doi.org/10.1016/j.ecoenv.2007.08.001

Yi Y, Xie B, Zhao T, Li Z, Stom D, Liu H (2019) Effect of external resistance on the sensitivity of microbial fuel cell biosensor for detection of different types of pollutants. Bioelectrochemistry 125:71–78. https://doi.org/10.1016/j.bioelechem.2018.09.003

Yu D, Bai L, Zhai J, Wang Y, Dong S (2017) Toxicity detection in water containing heavy metal ions with a self-powered microbial fuel cell-based biosensor. Talanta 168:210–216

Zhou T, Han H, Liu P, Xiong J, Tian F, Li X (2017) Microbial fuels cell-based biosensor for toxicity detection: a review. Sensors 17:2230

Zhu X, Logan BE (2014) Copper anode corrosion affects power generation in microbial fuel cells. J Chem Technol Biotechnol 89:471–474. https://doi.org/10.1002/jctb.4156